

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring lead in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify lead. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect lead in environmental samples are the methods approved by federal organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy, precision, and selectivity.

6.1 BIOLOGICAL SAMPLES

Blood, Urine, Serum, Cerebrospinal Fluid. There are several methods for the analysis of lead in biological samples. The most common methods currently used are flame atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry (GFAAS), anode stripping voltametry (ASV), inductively coupled plasma-atomic emission spectroscopy (ICP/AES), and inductively coupled plasma mass spectrometry (ICP/MS). Spectrophotometric methods also exist and were commonly used in the past; however, they are not as sensitive or reliable as the newer methods. According to Grandjean and Olsen (1984) and Flegal and Smith (1995), GFAAS and ASV are the methods of choice for the analysis of lead. In order to produce reliable results, background correction, such as Zeeman background correction that minimizes the impact of the absorbance of molecular species, must be applied. Limits of detection for lead using AAS are on the order of $\mu\text{g/mL}$ (ppm) and for GFAAS are generally in the low ng/mL (ppb) range (Flegal and Smith 1995). Other specialized methods for lead analysis are X-ray fluorescence spectroscopy (XRFS), neutron activation analysis (NAA), differential pulse anode stripping voltametry, and isotope dilution mass spectrometry (IDMS). The most reliable method for the determination of lead at low concentrations is IDMS (EPA 1986a; Grandjean and Olsen 1984), but due to the technical expertise required and high cost of the equipment, this method is not commonly used. It is primarily used for the development of certified standard reference materials by which other methods can determine their reliability since results of lead analyses from numerous laboratories often do not agree (Fell 1984). Details of several methods used for the analysis of lead in biological samples are presented in Table 6-1.

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Concentrations of lead in blood, urine, serum, and cerebrospinal fluid have been used as indicators of exposure to lead. Measurement of lead in blood is the most common method of assessing exposure. Blood lead is also considered the most useful tool for screening and diagnostic testing (Moore 1995); the half-life of lead in blood is approximately 36 days (Todd et al. 1996). A second half-life is generally considered to be approximately 4 years (Graziano 1994) and reflects the replenishment of lead in the blood from the bone storage compartment. Sample preparation usually consists of wet ashing (digesting) the sample with strong acid and heat, and redissolving the residue in dilute acid prior to analysis so that all lead species are converted quantitatively to the same lead compound (NIOSH 1977a, 1977d, 1977e, 1977g, 1977h). Preparation methods not requiring wet ashing have also been used with good results (Aguilera de Benzo et al. 1989; Delves and Campbell 1988; Manton and Cook 1984; NIOSH 1977f; Que Hee et al. 1985a; Zhang et al. 1997). For samples analyzed by ICP/MS, ASV, AAS, and GFAAS, sensitivity is in the low- to sub-ppb (0.1–15 ppb) with good accuracy and precision (Aguilera de Benzo et al. 1989; Delves and Campbell 1988; NIOSH 1977d, 1977e, 1977f, 1977g, 1977h; Que Hee et al. 1985a; Zhang et al. 1997). The presence of phosphate, ethylenediaminetetraacetic acid (EDTA) and oxalate can sequester lead and cause low readings in flame AAS (NIOSH 1984). A comparison of IDMS, ASV, and GFAAS showed that all three of these methods can be used to reliably quantify lead levels in blood (Que Hee et al. 1985a). ACGIH recommends quantification of blood lead by GFAAS. ESA, Inc., has introduced a simple to use, portable device for performing blood lead measurements using a finger stick or a venous sample (ESA 1998). Results can be obtained in about 3 minutes. For analysis of urine, chelation and solvent extraction, followed by atomic absorption for quantification is the recommended method (ACGIH 1986). Estimated accuracy reported for an IDMS technique was excellent (Manton and Cook 1984). Sensitivity and precision were not reported by the authors, but they are generally considered to be excellent (EPA 1986a; Grandjean and Olsen 1984).

In a recent article by Dyatlov et al. (1998), a method for the determination of Pb^{+2} and Ca^{+2} in intracellular fluids was described. In this method, a fluorescent, calcium indicator (fluo-3) was used. This dye fluoresces after binding Pb^{+2} and Ca^{+2} ; lead is considered an interferant to the determination of calcium by this approach. However, by complexing the divalent lead ion with the heavy metal chelator TPEN (N,N,N',N'-tetrakis(2-pyridylmethyl)ethylene-diamine) prior to the addition of the fluo-3, the fluorescent

Table 6-1. Analytical Methods for Determining Lead in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample limit detection	Percent recovery	Reference
Blood and tissue	Digestion of sample with HNO_3 / HClO_4 / H_2SO_4 ; heat	ICP/AES (Method P&CAM 8005)	0.01 $\mu\text{g/g}$ (blood) 0.2 $\mu\text{g/g}$ (tissue)	113	NIOSH 1985a
Blood	Addition of 50 μL of blood into reagent, mixing, and transferring to sensor strip (commercial test kit)	Gold electrode sensor	1.4 $\mu\text{g/dL}$	No data	ESA 1998
Urine	Extraction of sample with polydithio-carbamate resin and NaOH ; filtration on cellulose ester membrane; neutralization with NaOH ; ashing; dissolution and heating; dilution with distilled water	ICP/AES (Method P&CAM 8310)	0.005 $\mu\text{g/mL}$	100	NIOSH 1984
Serum blood, and urine	Filtration of sample if needed; blood requires digestion in a Parr bomb; dilution of serum or urine with acid or water	ICP/AES	10–50 $\mu\text{g/L}$	85 (serum) >80 (urine, blood)	Que Hee and Boyle 1988
Urine	Wet ashing of sample with acid mixture and dissolution in dilute HClO_4	ASV with mercury-graphite electrode (Method P&CAM 200)	4 $\mu\text{g/L}$	90–110	NIOSH 1977e
Urine (δ -aminolevulinic acid)	Dilution of sample; reaction with ethylacetoacetate and ethylacetate to form δ -amino-levulinic acid-pyrrole; reaction with Erlich's reagent	Spectrophotometry	No data	No data	Tomokuni and Ichiba 1988
Urine (δ -aminolevulinic acid)	Acidification of sample; separate δ -aminolevulinic acid on HPLC; reaction with formaldehyde and acetylacetone	HPLC/FL	10 $\mu\text{g/L}$	No data	Tabuchi et al. 1989
Plasma, Urine (δ -aminolevulinic acid)	Derivatization of δ -aminolevulinic acid with formaldehyde and acetylacetone to form fluorescent compounds; separation using HPLC	HPLC/FL	3 $\mu\text{g/L}$	No data	Oishi et al. 1996

Table 6-1. Analytical Methods for Determining Lead in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample limit detection	Percent recovery	Reference
Serum and cerebrospinal fluid	²⁰⁶ Pb addition and sample acid digestion; lead isolation by ion-exchange, elution, and deposition onto platinum wire	IDMS	No data	80–120	Manton and Cook 1984
Feces	Dessication and pulverization of sample; digestion with hot acid in Paar bomb	ICP/AES	10–50 µg/L	>86	Que Hee and Boyle 1988
Testes, liver, spleen, kidney	Dicing of sample and digestion in hot acid in a Paar bomb; evaporation; redissolution in HCl/HNO ₃	ICP/AES	10–50 µg/L	>80	Que Hee and Boyle 1988
Spleen, liver, and kidney; Liver, kidney, muscle	Wet digestion of sample with HNO ₃ -HClO ₄ mixture; Bomb digestion of sample with acid and heat or digestion with acid and dry ashing; dissolution in acid; dilution with water	GFAAS	No data	No data	Blakley and Archer 1982; Ellen and Van Loon 1990
		GFAAS	20 µg/g (bomb); 5 µg/g (dry ashing)	85–107 (bomb); 75–107 (dry ashing)	
		DPASV	No data	82–120	
Tissues (brain, heart, lung, kidney, liver, and testes)	Dry ashing of sample; dissolution in HNO ₃	AAS	No data	No data	Exon et al. 1979
Tissues	Freeze drying of samples; subjection to thermal neutron irradiation; chemical separation of elements	NAA	No data	No data	Hewitt 1988
Brain	Wet ashing of sample with mixture of acids, mixing with Metex® and analysis	ASV	No data	No data	Jason and Kellogg 1981
Bone	Partially polarized photon directed at second phalanx of left forefinger (noninvasive technique)	K-XRF	20 µg/g	No data	Christoffersson et al. 1986

Table 6-1. Analytical Methods for Determining Lead in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample limit detection	Percent recovery	Reference
Bone	Partially polarized photon directed at anteromedial skin surface of mid-tibia (non-invasive technique)	L-XRF	20 µg/g	No data	Wielopolski et al. 1986
Teeth	Cleaning and sectioning of tooth; digestion with HNO ₃ ; evaporation; redissolution in buffer solution	ASV	No data	83–114	Rabinowitz et al. 1989
Teeth	Dry ashing of sample; crushing; dry ashing again; dissolution in HNO ₃	AAS	No data	90–110	Steenhout and Pourtois 1981
Hair	Cleaning of sample with acetone/ methanol; digestion with acid mixture and heat; diammonium phosphate addition as matrix modifier	GFAAS	0.16 µg/g	99	Wilhelm et al. 1989
Bone	¹⁰⁹ Cd gamma-ray irradiation with source at 2.5 cm from skin of proximal tibia	K-XRF	2 µg/g	No data	Hu et al. 1989, 1990, 1991
Hair	Cleaning of sample with hexane, ethanol, and water; wet ashing with HNO ₃ and H ₂ O ₂	ICP/AES	No data	No data	Thatcher et al. 1982

AAS = atomic absorption spectroscopy; APDC = ammonium pyrrolidine dithiocarbamate; ASV = anode stripping voltammetry; Ba(NQ)₂ = barium nitrate; ¹⁰⁹Cd = cadmium 109 radioisotope; DPASV = differential pulse anodic stripping voltammetry; GFAAS = graphite furnace atomic absorption spectroscopy; H₂O₂ = hydrogen peroxide; HCl = hydrogen chloride; H₂SO₄ = sulfuric acid; HClO₄ = perchloric acid; HNO₃ = nitric acid; HPLC/FL = high performance liquid chromatography/fluorimetry; ICP/AES = inductively coupled plasma/atomic emission spectroscopy; ICP-MS = inductively coupled plasma-mass spectrometry; IDMS = isotope dilution mass spectrometry; K-XRF = K-wave X-ray fluorescence; L-XRF = L-wave X-ray fluorescence; MIBK = methyl isobutyl ketone; NAA = neutron activation analysis; NaOH = sodium hydroxide; NIOSH = National Institute for Occupational Safety and Health; P&CAM = physical and chemical analytical methods; ²⁰⁶Pb = lead 206

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product was proportional to the concentration of Ca^{+2} and lead was determined by difference. An LOD of 1 pM lead was estimated for cell-free media. This interaction shows how divalent lead can interfere with the actions of other divalent cations such as calcium, an aspect of crucial importance in living organisms (see Chapter 2).

Several biomarkers exist for monitoring exposure to lead. A number of biochemical assays are available for the assessment of lead exposure and toxicity in the human body using standard clinical laboratory techniques. Details of such assays are reported in several reviews (EPA 1986a; Grandjean and Olsen 1984; Stokinger 1981) and are also available in standard clinical laboratory methods manuals. The commonly used assays are coproporphyrin, 1,25-dihydroxyvitamin D, ALA (δ -aminolevulinic acid), and EP (erythrocyte protoporphyrin) concentrations and ALAD (ALA dehydratase) activity. All of these assays are sensitive, reliable, and well established; however, erythrocyte protoporphyrin and ALAD activity appear to be the most useful and sensitive for determining exposure to lead. A recent review (Porru and Alessio 1996) indicated that ALAD activity was proportional to blood lead concentration ranging from 10–40 $\mu\text{g/dL}$, and EP concentration was proportional to blood lead over the range of 30–80 $\mu\text{g/dL}$. The EP concentration was said to be useful for assessing exposure experienced over the past 3 to 4 months. Urinary ALA, however, was not proportional to blood lead until the blood concentrations reached 60–70 $\mu\text{g/dL}$, a concentration too high to be of use for early screening since other clinical symptoms should already be evident. A colorimetric method for detection of ALA in urine, in which the pyrrole from ALA is formed and reacted with Ehrlich's reagent to form a colored end product, has been used successfully (Tomokuni and Ichiba 1988). ALA has also been determined in urine using high-performance liquid chromatography (HPLC) followed by quantification of a fluorescent end product (Tabuchi et al. 1989). A similar approach to ALA determination in blood and urine was described by Oishi et al. (1996) and was more sensitive than the method of Tabuchi et al. (1989). Erythrocyte protoporphyrin bound to zinc has been quantified using hemofluorimetry (Braithwaite and Brown 1987). An HPLC/fluorescent method has been reported for determination of coproporphyrin in urine (Tomokuni et al. 1988). Other biological assays that have been used as indicators of lead exposure are serum immunoglobulins and salivary IgA (Ewers et al. 1982). While all these biological assays are reliable and have been verified for clinical laboratory use, they are not specific for lead.

Tissues. Lead has been quantified in a variety of tissues, including liver, kidney, brain, heart, lung, muscle, and testes. Techniques for measuring lead in tissues are similar to those used for blood and urine. When AAS, GFAAS, or ASV are used for analysis, the samples may be wet ashed, digested with acid, or

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bomb digested (Blakley and Archer 1982; Blakley et al. 1982; Ellen and Van Loon 1990; Exon et al. 1979; Jason and Kellogg 1981; Que Hee and Boyle 1988). The information located did not allow an adequate comparison between these methods. Parr bomb digestions are recommended for estimation of metals in biological tissues (Que Hee and Boyle 1988). Sensitivities reported for GFAAS and ICP/AES are in the low ppm (5–20 ppm) (Ellen and Van Loon 1990) and are probably comparable for the other techniques. Differential anodic stripping pulse voltametry (DPASV) and NAA have also been used to analyze tissues for lead. Sample preparation for DPASV is the same as those for AAS, GFAAS, and ASV. Its accuracy and precision are comparable to results using GFAAS, and its sensitivity is slightly greater (Ellen and Van Loon 1990). Determination of lead in tissue samples following freeze drying, neutron irradiation, and chemical separation has been reported. An advantage of this method is that the sample does not have to be dissolved. No further information was reported for the method (Hewitt 1988).

Hair, Teeth, and Bone. Noninvasive methods using X-ray fluorescence can be used for the determination of lead concentration in bones. These methods include L X-rays of the tibia using an X-ray generator (Wielopolski et al. 1986); K X-rays in the second phalanx of the index finger using a cobalt source and a germanium silicon detector (Christoffersson et al. 1986); and *in vivo* tibial K X-ray fluorescence (Batuman et al. 1989; Hu et al. 1989, 1990, 1991). This latter method has the advantage of deeper penetration of the bone (2 cm) to allow for averaging lead concentrations over the whole bone thickness (Wedeen 1990). The better penetration also alleviates errors resulting from the measurement of overlying skin and makes the method relatively insensitive to movement of the subject during the 15-minute sampling period (Landigran and Todd 1994). The level of lead in bone has been reported to be a good indicator of stored lead in body tissue (Ahlgren et al. 1976; Bloch et al. 1976; Rosen et al. 1987; Skerfving et al. 1993). The sensitivity of the technique is in the low ppm and the precision is acceptable. Advantages are that no sample preparation is required and the technique can safely and easily be done on live subjects. Teeth have been analyzed for lead using AAS and ASV (Rabinowitz et al. 1989; Steenhout and Pourtois 1981). Samples must be dry ashed or digested with acid prior to analysis. Precision and accuracy of both AAS and ASV are good. Detection limits were not reported by the authors. A detection limit in the sub-ppm (0.16 ppm) and high accuracy were reported for GFAAS analysis of hair samples (Wilhelm et al. 1989). ICP/AES has also been used to analyze hair for lead, but lack of data prevents a comparison with the AAS method (Thatcher et al. 1982).

The isotopic distribution of lead (IDMS) in shed teeth from children has been shown to be useful in studies of the history of exposure to lead, including the definition of the source of the exposure, e.g., mine dust vs.

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food (Gulson and Wilson 1994), so IDMS certainly has important applicability, if not for routine determinations. ICP/MS, however, is easier, more sensitive, allows for multi-element analysis, and provides isotopic data.

6.2 ENVIRONMENTAL SAMPLES

The primary methods of analyzing for lead in environmental samples are AAS, GFAAS, ASV, ICP/AES, and XRFs (Lima et al. 1995). Less commonly employed techniques include ICP/MS, gas chromatography/photoionization detector (GC/PID), IDMS, DPASV, electron probe X-ray microanalysis (EPXMA), and laser microprobe mass analysis (LAMMA). The use of ICP/MS will become more routine in the future because of the sensitivity and specificity of the technique. ICP/MS is generally 3 orders of magnitude more sensitive than ICP/AES (Al-Rashdan et al. 1991). Chromatography (GC, HPLC) in conjunction with ICP/MS can also permit the separation and quantification of organometallic and inorganic forms of lead (Al-Rashdan et al. 1991). In determining the lead concentrations in the atmosphere and water, a distinction between the concentration of lead in the particulate and gaseous or dissolved forms is often necessary. Particulate lead can be separated from either media using a filter technique. The filter collects the particulate matter and allows the dissolved material to pass through for separate analysis of each form. As with the analysis of biological samples, the definitive method of analysis for lead is IDMS. However, most laboratories do not possess the expertise or equipment required for this method. ICP/MS is becoming more available and will probably soon become the major method. Table 6-2 summarizes several methods for determining lead in a variety of environmental matrices.

Air. Various methods have been used to analyze for particulate lead in air. The primary methods, AAS, GFAAS, ICP/AES are sensitive to levels in the low $\mu\text{g}/\text{m}^3$ range ($0.1\text{--}20 \mu\text{g}/\text{m}^3$) (Birch et al. 1980; EPA 1988b; NIOSH 1977c, 1977g, 1981, 1984, 1994; Scott et al. 1976). Accuracy and precision are generally good. GFAAS is considered to be more sensitive than AAS; however, AAS is not subject to as much interference from matrix effects as GFAAS (NIOSH 1977b, 1977g, 1977i). Detection of particulate lead by generation of the lead hydride has been used to increase the sensitivity of the AAS technique (Nerin et al. 1989). Excellent accuracy and precision was reported for this method. ASV has

Table 6-2. Analytical Methods for Determining Lead in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (particulate lead)	Collection of particulate matter onto membrane filter; digestion with HNO_3 / H_2O_2 ; dilution with distilled water	GFAAS (Method P&CAM 7105)	$2.0 \mu\text{g}/\text{m}^3$	82–103	NIOSH 1990, 1994
Air (particulate lead)	Collection of particulate matter onto membrane filter; wet ashing with HNO_3 / HClO_4 / H_2SO_4 ; dissolution in acetate buffer	ASV with mercury-graphite electrode (Method P&CAM 191)	$0.16 \mu\text{g}/\text{m}^3$	90–110	NIOSH 1977c
Air (particulate lead)	Collection of particulate matter onto membrane filter; wet ashing with HNO_3	AAS flame (Method 7082)	$50 \mu\text{g}/\text{m}^3$	82–103	NIOSH 1984
Air (particulate lead)	Collection of particulate matter onto cellulose acetate membrane filter; wet ashing with HNO_3 / HClO_4	ICP/AES (Method P&CAM 7300)	$5 \mu\text{g}/\text{m}^3$	95–105	NIOSH 1984
Air (particulate lead)	Collection of particulate matter onto filter; extraction with HNO_3 / HCl , heat, and sonication	ICP/AES	No data	No data	EPA 1988a
Air (particulate lead)	Collection of particulate matter onto filter; dry ashing; extraction with HNO_3 / HCl ; dilution with HNO_3	AAS AES	$0.1 \mu\text{g}/\text{m}^3$ $0.15 \mu\text{g}/\text{m}^3$	93 102	Scott et al. 1976
Air (particulate lead)	Collection of sample onto cellulose acetate filter; dissolution in HNO_3 with heat; addition of HCl / H_2O_2 and reaction in hydride generator with sodium borohydride to generate lead hydride	AAS	$8 \text{ ng}/\text{L}$	100–101	Nerin et al. 1989
Air (particulate lead)	Collection of sample onto filter; addition of ^{206}Pb to filter; dissolution of filter in NaOH ; acidification; separation of lead by electrodeposition; dissolution in acid	IDMS	$0.1 \text{ ng}/\text{m}^3$	No data	Volkening et al. 1988
Air (particulate PbS)	Collection of particles onto filter, suspension in THF, recollection onto silver filter	XRD	$60 \mu\text{g}/\text{m}^3$	102.6	NIOSH 1994
Air (particulate lead)	Collection of sample onto nucleopore polycarbonate filter; coating of filter sections with carbon	EPXMA LAMMA	No data No data	No data No data	Van Borm et al. 1990
Air (tetramethyl and tetraethyl lead)	Adsorption of volatile compounds in filtered sample onto XAD-2 resin, desorption with pentane	GC/PID (Method 2534 (TML) and 2537 (TEL))	$40 \mu\text{g}/\text{m}^3$ (TML) $45 \mu\text{g}/\text{m}^3$ (TEL)	99 105.5	NIOSH 1987
Air (particulate and organolead)	Collection of particulate matter collected onto glass fiber filter; passage of filtered gases through iodine monochloride bubblers; wet ashing of particulate matter; conversion of lead compounds in bubbler solution to dithiazone complex in presence of EDTA-salts and extraction with carbon tetrachloride solution followed by acid extraction	GFAAS	No data (particulate); $0.25 \text{ ng}/\text{m}^3$ (gaseous)	No data (particulate); 95–99 (gaseous)	Birch et al. 1980

Table 6-2. Analytical Methods for Determining Lead in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (particulate and organolead)	Collection of particulate matter collected onto nucleopore filters; filtered gases cryogenically trapped and thermally desorbed	XRF (particulate) GC/GFAAS (gaseous)	0.3 µg/m ³ 0.2 ng/m ³	46→90 90–100	De Jonghe et al. 1981
Surface contamination (lead and its compounds)	Wiping of defined area surface using a moistened gauze pad; digestion of sample using nitric acid; dilution.	ICP/AES GFAAS	2 µg/sample 0.1 µg/sample	No data	NIOSH 1994
Water (particulate and dissolved lead)	Filtration of water through a 0.45 µm membrane filter (dissolved lead); particulate material dissolved by wet ashing (insoluble lead)	ICP/AES (EPA Method 200.7)	42 µg/L	94–125	EPA 1983a
Water (TAL)	Extraction with hexane	GC/AAS	0.5 µg/L	88–90	Chau et al. 1979
Water (TAL)	Purging of sample with gas followed by cryogenically trapping volatile species onto solid sorbent GC column	GC/AAS	0.5 ng/g	No data	Chau et al. 1980
Water (alkyl lead)	Complexation of sample with diethyldithiocarbamate; extraction with pentane; removal of water; butylation; extraction with nonane	GC/AAS	1.25 ng/L	90–108	Chakraborti et al. 1984
Water (particulate and dissolved lead)	Filtration of water through a 0.45 µm membrane filter (dissolved lead); particulate material dissolved by wet ashing (insoluble lead)	AAS (EPA Method 239.1) GFAAS (EPA Method 239.2)	0.1 mg/L 1 µg/L	99.8–125.7 88–95	EPA 1983a
Water (total lead)	Digestion of sample with acid and heat; dilution with water	AAS	1.0 ng/g	No data	Chau et al. 1979
Water (dissolved or total)	Digestion of water sample followed by filtration, acidification, addition of ammoniacal citrate-cyanide reducing solution; extraction with chloroform containing dithizone.	Measure absorbance at 510 nm (Standard Method 3500-PbD)	0.5 µg/L	98.6% at 10.4 µg/L (6.8% RSD)	Eaton et al. 1995a
Water	Direct introduction of water or of extract following extraction of water with methyl isobutyl ketone containing ammonium pyrrolidine dithiocarbamate.	AAS (Standard Method 3111)	0.5 mg/L at 283.3 nm	No data (%RSD = 4.7 for direct; 23.5 for extract)	Eaton et al. 1995b
Water and wastewater (dissolved, total)	Addition of matrix modifier, analysis	GFAAS (Standard Method 3113)	1 µg/L at 283.3 nm	117% at 10.4 µg/L (31 %RSD for surface water at 10.4 µg/L)	Eaton et al. 1995c

Table 6-2. Analytical Methods for Determining Lead in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water and wastewater (dissolved, total)	Filtration/acidification and analysis for dissolved; digestion followed by analysis for total	ICP/AES (Standard Method 3120)	40 µg/L	104% (12.5 %RSD) at 100 µg/L	Eaton et al. 1995d
Water, extracts or digests of waste	Filtration or digestion as appropriate (depends on matrix, dissolved or total, acid leachable, etc.)	ICP/MS (EPA Method 6020)	No data	71–137% (11–23% RSD) for aqueous solutions; 90–104% (6–28% RSD) for solid samples	EPA 1994e
Water	Filtration; addition of $\text{Ni}(\text{NO}_3)_2$ and $\text{NH}_4\text{H}_2\text{PO}_4$ matrix modifiers	ETAAS	0.14 µg/L	89–101	Xu and Liang 1997
Water (total lead)	Filtration of sample followed by analysis; digestion of filter with acid	ICP/AES	10–50 µg/L	>80	Que Hee and Boyle 1988
Soil	Drying of soil sample followed by sieving; digestion with HNO_3 ; centrifugation	ICP/AES	0.09 µg/g	97–103	Schmitt et al. 1988
Dust	Wiping of hard surface of known dimension; acid digestion	ICP/AES AAS GFAAS	Varies	No data	ASTM 1998f (ASTM E 1728) ASTM 1998b (ASTM E 1644) ASTM 1998a (ASTM E 1613)
Soil	Drying of soil followed by homogenization, digestion with nitric acid and hydrogen peroxide, dilution	ICP/AES AAS GFAAS	Varies	No data	ASTM 1998e (ASTM E 1727) ASTM 1998d (ASTM E 1726) ASTM 1998a (ASTM E 1613)
Soil	Drying of soil sample followed by sieving, digestion with HNO_3 , filtration	AAS	no data	no data	Mielke et al. 1983
Soil	Drying of sample and sieving for XRF; digestion of sieved sample with HNO_3 and heat for AAS	XRF AAS	No data No data	65–98 63–68	Krueger and Duguay 1989
Soil	Drying of sample, dry ashing, digestion with acid, and dilution with water	AAS	2 µg/g	79–103	Beyer and Cromartie 1987

Table 6-2. Analytical Methods for Determining Lead in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Digestion with HNO ₃ and H ₂ O ₂ ; evaporation; redissolution with HNO ₃ ; filtration	FI-HG-AAS	2 µg/L	98–101	Samanta and Chakraborti 1996.
Soil, wastes, and groundwater	Acid digestion of sample, dilution with water, and filtration	AAS (EPA method 7420)	0.1 mg/L	No data	EPA 1986e
		GFAAS (EPA method 7421)	1 µg/L	No data	
Soil, dust, and paint	Digestion of sample with hot acid; evaporation of water; redissolution in HNO ₃	AAS	12 ng/g	>80	Que Hee et al. 1985b
Sediment	Digestion of sample with hot HNO ₃ /H ₂ SO ₄	GFAAS	No data	92–95	Bloom and Crecelius 1987
Sediment, fish (TAL)	Homogenization of fish; addition of EDTA to sample; extraction with hexane; centrifugation; isolation off organic layer for analysis.	GC/AAS	0.01 µg/g (sediment) 0.025 µg/g	81–85 72–76	Chau et al. 1979
Sediment, (fish), vegetation (TAL)	Purging of sample with gas followed by cryogenically trapping volatile species onto solid sorbent GC column.	GC/AAS	0.1 ng/g (solid)	No data	Chau et al. 1980
Sediment, fish, vegetation (total lead)	Digestion of sample with acid and heat; dilution with water	AAS	50 ng/g (sediment)	No data	Chau et al. 1980
			10 ng/g (fish and vegetation)	No data	
Dried paint	Sample collection using heat gun, cold scraping, or coring methods; microwave digestion with nitric acid and hydrochloric acid	ICP/AES AAS GFAAS	Varies	No data	ASTM 1998g (ASTM E 1729) ASTM 1998c (ASTM E 1645) ASTM 1998a (ASTM E 1613)
Milk	Addition of 50 µL (C ₂ H ₅) ₄ NOH in ethanol to 25 µL milk followed by heating and dilution with water to 125 µL	GFAAS	No data	No data	Michaelson and Sauerhoff 1974
Evaporated milk	Dry ashing of sample; dissolution in HNO ₃	ASV	0.005 µg/g	99	Capar and Rigsby 1989
Mussel, tomato	Digestion of sample with acid or acid plus catalyst; generation of lead hydride	GFAAS	4 ng/g	94–95	Aroza et al. 1989
Agricultural crops	Dry ashing of sample with H ₂ SO ₄ and HNO ₃ ; dilution with water	DPASV	0.4 ng/g	85–106	Satzger et al. 1982

Table 6-2. Analytical Methods for Determining Lead in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Grains, milk mussel, fish	Bomb digestion of sample with acid and heat or digestion with acid and dry ashing; dissolution in acid; dilution with water	GFAAS	20 µg/g (bomb); 5 µg/g (dry ash) No data	85–107	Ellen and Van Loon 1990
				75–107	
				82–120	
Edible oils	Microwave digestion with acid mixture; (NH ₄) ₂ PO ₄ added as matrix modifier	DPASV		82–120	Allen et al. 1998
		ICP/AES	50 ng/g	75–107	
Citrus leaves and paint	Chopping or pulverization of sample; digestion with hot acid; evaporation of water; redissolution in acid	GFAAS	30 ng/g	78–117	Que Hee and Boyle 1988
		ICP/AES	10–50 µg/L	75–82 (citrus leaves);	
				89–96 (paint)	

AA = atomic absorption; AAS = atomic absorption spectroscopy; AES = atomic emissions spectroscopy; ASV = anode stripping voltammetry; (C₂H₅)₄NOH = tetraethylammonium hydroxide; DPASV = differential pulse anodic stripping voltammetry; EDTA = ethylenediamine tetraacetic acid; EPA = Environmental Protection Agency; EPXMA = electron probe X-ray micro-analysis; ETAAS = electrothermal atomic absorption spectroscopy; GC = gas chromatography; GFAAS = graphite furnace atomic absorption spectrometry; HCl = hydrochloric acid; HClO₄ = perchloric acid; HNO₃ = nitric acid; H₂O₂ = hydrogen peroxide; H₂SO₄ = sulfuric acid; ICP/AES = inductively coupled plasma-atomic emission spectroscopy; IDMS = isotope dilution mass spectrometry; LAMMA = laser microprobe mass analysis; MS = mass spectrometry; NaOH = sodium hydroxide; NG = nanogram; ²⁰⁶Pb = lead 206; P&CAM = physical and chemical analytical methods; PID = photoionization detector; TAL = tetraalkyl leads; TEL = tetraethyl lead; THF = tetrahydrofuran; TML = tetramethyl lead; XRD = X-ray diffraction; XRF = X-ray fluorescence

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a wide range as well as high sensitivity. It is relatively inexpensive compared to other methods (NIOSH 1977b). Advantages of ICP/AES are that it has a wide range and allows analysis of several elements at once. However, the technique is expensive in terms of equipment and supplies (NIOSH 1981). XRFs has been used to analyze for particulate lead in air (DeJonghe et al. 1981). While sensitivity was good, recovery was highly variable and relatively low compared to other methods. The highest sensitivity was obtained with IDMS, as expected (Volkening et al. 1988). As previously stated, this is the definitive method for determining lead in environmental, as well as biological samples. Two sophisticated methods, EPXMA and LAMMA, have been used to determine the inorganic lead species present in particulate matter in air (Van Borm et al. 1990).

Determination of lead vapor in air requires prior filtering of the air to exclude particulate lead, and trapping of the gaseous components. Gaseous lead is also referred to as organic lead or alkyl lead, the most common being the tetraalkyl species. Organic lead species may be trapped by liquid or solid sorbents, or cryogenically (Birch et al. 1980; DeJonghe et al. 1981; NIOSH 1978b). Gas chromatography (GC) is used to separate the different alkyl species. Detection by GFAAS and PID have been reported (DeJonghe et al. 1981; NIOSH 1978b). GFAAS detection is more sensitive than PID, but both have good accuracy.

Water. As with air, water can be analyzed for both particulate and dissolved (organic) lead. Particulate lead collected on a filter is usually wet ashed prior to analysis. Comparison of the GFAAS and AAS methods for particulate lead showed the former technique to be about 100 times more sensitive than the latter, although both offer relatively good accuracy and precision (EPA 1983). ICP/MS has been used to determine lead in water (EPA 1994e). Chelation/extraction can also be used to recover lead from aqueous matrices (Eaton 1995b). GC/AAS has been used to determine organic lead, present as various alkyl lead species, in water (Chakraborti et al. 1984; Chau et al. 1979, 1980). Sample preparation for organic lead analysis was either by organic solvent extraction (Chakraborti et al. 1984; Chau et al. 1979) or purge-and-trap (Chau et al. 1980). Sensitivity was in the ppb to ppt range and reliability was similar for all three methods. Total lead can be determined by digesting samples with acid and analyzing by either AAS or the more sensitive GFAAS (Chau et al. 1980; EPA 1982c, 1986e).

Dusts, Sediments, and Soil. Both total and organic lead have been determined in dusts, sediments, and soils. In most cases the sample must be digested with acid to break down the organic matrix prior to analysis (ASTM 1998b, 1998d; Beyer and Cromartie 1987; Bloom and Crecelius 1987; EPA 1982c, 1986e; Krueger and Duguay 1989; Mielke et al. 1983; Que Hee and Boyle 1988; Que Hee et al. 1985b;

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Samanta and Chakraborti 1996; Schmitt et al. 1988); however, organic extraction (Chau et al. 1979) and purge-and-trap (Chau et al. 1980) have also been used. The primary detection methods are ICP/AES, AAS or GFAAS, GFAAS being more sensitive, but also more susceptible to interference. When quantification of organic lead is desired, GC is employed to separate the alkyl lead species (Chau et al. 1979, 1980). Precision and accuracy are acceptable for these atomic absorption-based methods (Beyer and Cromartie 1987; Bloom and Crecelius 1987; Chau et al. 1979; EPA 1982c, 1986e; Krueger and Duguay 1989; Que Hee et al. 1985b). ICP/AES is reported to be more sensitive and reliable than atomic absorption techniques (Schmitt et al. 1988), but sample collection and preparation methods have been shown to strongly influence the reliability of the overall method (Que Hee et al. 1985b). Sampling of house dust and hand dust of children requires special procedures (Que Hee et al. 1985b). XRFS appears to provide a simpler method of measuring lead in soil matrices; however, the available data do not permit an assessment of the techniques sensitivity and reliability for soil analysis (Krueger and Duguay 1989). XRFS has been shown to permit speciation of inorganic and organic forms of lead in soil for source elucidation (Manceau et al. 1996).

Other Matrices. Lead has been determined in several other environmental matrices, including paint, fish, vegetation, agricultural crops, and various foods. As with soil, the methods of choice are either ICP/AES, AAS, or GFAAS. Samples may be prepared using one of the methods described for sediment and soil or by wet or dry ashing (Aroza et al. 1989; ASTM 1998d; Capar and Rigsby 1989; Que Hee and Boyle 1988; Que Hee et al. 1985b; Satzger et al. 1982). ASV and DPASV have also been used with good sensitivity (ppb) and reliability to analyze for lead in other environmental media (Capar and Rigsby 1989; Ellen and Van Loon 1990; Satzger et al. 1982).

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of lead is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of lead.

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The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect Methods are available for measuring inorganic lead in blood, serum, urine, cerebrospinal fluid, tissues, bone, teeth, and hair (Aguilera de Benzo et al. 1989; Batuman et al. 1989; Blakley and Archer 1982; Blakley et al. 1982; Christoffersson et al. 1986; Delves and Campbell 1988; Ellen and Van Loon 1990; Exon et al. 1979; Hu et al. 1989, 1990, 1991; Jason and Kellogg 1981; Manton and Cook 1984; NIOSH 1977a, 1977d, 1977e, 1977f, 1977g, 1977h, 1984; Que Hee and Boyle 1988; Que Hee et al. 1985a; Wielopolski et al. 1986; Zhang et al. 1997). Available methods for determining lead in body fluids are sensitive and reliable for measuring background exposure levels, as well as exposure levels at which health effects have been observed to occur. Blood lead levels have been found to correlate best with exposure concentrations (Rabinowitz et al. 1985; Moore 1995). Methods of quantifying lead in tissues, bone, teeth, and hair are generally reliable, but are only sensitive at relatively high exposure concentrations. There is a need for more sensitive methods of detection for matrices so that correlations between lead levels in these media and exposure concentrations can be more reliably determined. Several nonspecific biomarkers are used to assess exposure to lead. These include ALAD activity and ALA, EP, coproporphyrin, and 1,25-dihydroxyvitamin D concentrations (Braithwaite and Brown 1987; EPA 1986a; Grandjean and Olsen 1984; Oishi et al. 1996; Porru and Alessio 1996; Stokinger 1981; Tabuchi et al. 1989; Tomokuni and Ichiba 1988; Tomokuni et al. 1988). The methods for determining these variables are sensitive, reliable, and well established. No additional research for these biomarkers appears to be needed. There is a need to identify and quantify those molecules responsible for lead transport within the body; the measurement of lead associated with these compounds could provide additional information about exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Numerous analytical methods are available for measuring inorganic and organic lead compounds in air, water, sediments, dust, paint, soil, fish, agricultural products, and foodstuffs (Eaton et al. 1995a, 1995b, 1995c, 1995d; Eckel and Jacob 1988; EPA 1982a, 1986a, 1988b, 1988f, 1989f, 1989h,

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1990c, 1994e; Lee et al. 1989; Maenhaut et al. 1979; Mielke 1992; Mielke et al. 1983, 1985, 1989). Most of these are sensitive and reliable for determining background concentrations of lead compounds in the environment and levels at which health effects might occur. The most frequently used methods are AAS, GFAAS, ASV, and ICP/AES, the methods recommended by EPA and NIOSH (ASTM 1998a; Birch et al. 1980; EPA 1988b; NIOSH 1977c, 1981, 1984; Scott et al. 1976). The definitive method is IDMS, which is used to produce reference standards by which laboratories can determine the reliability of their analyses (Volkening et al. 1988). No additional analytical methods for determining low levels of lead compounds in environmental media are needed. Additional method development work is needed if individual lead species in environmental media are to be accurately determined. ICP/MS based methods should be critically examined.

6.3.2 Ongoing Studies

Ongoing studies regarding analytical methods for lead were reported in the Federal Research in Progress File (FEDRIP 1998) database. Only one had relevance to analytical methods and was related to biomarkers. Dr. Liebelt at Yale University, with funding from the National Center for Research Resources, is investigating erythropoietin production in children with lead poisoning.